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Pharmacokinetics and Pharmacodynamics of once-daily prolonged-release tacrolimus in liver transplant recipients

Running Title: Pharmacokinetics and Pharmacodynamics of once-daily prolonged-release tacrolimus

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1 **Abstract**

2 **Purpose**

3 There is limited published data regarding the pharmacokinetics (PK) and pharmacodynamics
4 (PD) of prolonged-release tacrolimus (PRT) after liver transplant. We aimed to compare PK
5 and PD of PRT in early and stable liver transplant recipients by developing a population PK
6 model of PRT and investigating the profile of calcineurin activity (CNA) in the peripheral
7 blood mononuclear cells.

8 **Methods**

9 A conversion from twice-daily immediate-release tacrolimus (IRT) to once-daily PRT based
10 on one-to-one daily dose was performed at day 7 (D7) and D90 post-transplantation in
11 groups A ($n = 12$) and B ($n = 12$), respectively. Extensive PK samplings including whole blood
12 tacrolimus (TAC) concentration and CNA assessment were performed at D14 and D104 in
13 groups A and B, respectively. TAC concentration-time data ($n = 221$) were analyzed using
14 non-linear mixed effects modeling.

15 **Findings**

16 A two-compartment model with linear elimination and a delayed first order absorption
17 characterized by two transit compartments best described PK data. Model-predicted dose-
18 normalized (6.0 mg/day) area under the TAC concentration-time curve over the dosing
19 interval (AUC_{TAC}) in groups A and B were similar (geometric mean 235.6 ng/mL.h [CI95% =
20 139.6 – 598.7] vs 224.6 ng/mL.h [117.6 – 421.5], respectively, $p = 0.94$). Area under the CNA
21 versus time curve over the dosing interval (AUC_{CNA}) were not different between both groups
22 (4897 ± 3437 and 4079 ± 1008 pmol/min/ 10^6 cells, respectively, $p = 0.50$). In group A, trough
23 CNA at D14 post-transplantation was statistically higher than that measured just before the
24 switch to PRT (i.e D7 post-transplantation) (198 ± 92 vs 124 ± 72 pmol/min/ 10^6 cells, $n = 8$,
25 respectively, $p = 0.048$), while no statistical difference in TAC concentration was observed (p
26 = 0.11). In group B, no statistical difference between D90 and D104 was observed in either
27 trough CNA (149 ± 78 vs 172 ± 82 pmol/min/ 10^6 cells, respectively, $n = 6$, $p = 0.18$) or TAC
28 concentration ($p = 0.17$). No graft rejection was observed in either of the groups.

1 **Implications**

2 This study suggests that one-to-one dosage conversion to once-daily PRT during the early
3 post-transplantation period could result in significant CNA variations but without causing
4 graft rejection. Further investigations in larger cohorts are warranted to confirm these
5 results.

6 Study registry identification number: ClinicalTrials.gov Registration identification
7 NCT02105155

8

9 **Keywords:** liver transplantation; prolonged-release tacrolimus; pharmacokinetics;
10 calcineurin activity

1 **1. Introduction**

2 Tacrolimus (TAC) is a key immunosuppressive agent for the prevention and treatment of
3 allograft rejection in liver transplantation¹. TAC binds with high affinity to FK-binding protein
4 12². The drug-receptor complex specifically and competitively binds to and inhibits
5 calcineurin, a calcium- and calmodulin-dependent phosphatase. This process inhibits the
6 translocation of a family of transcription factors (NF-AT), leading to reduced transcriptional
7 activation of cytokine genes such as interleukin (IL)-2 and thereby to a reduction of T-cell
8 proliferation³. TAC has a narrow therapeutic range and a significant between-subject
9 variability (BSV), and thus a close monitoring of whole blood trough concentrations is
10 required to avoid under- or over-exposure⁴. Hence, therapeutic drug monitoring of TAC in
11 liver transplant recipients is the benchmark method in this indication¹. However, some liver
12 transplant recipients with sufficient exposure to TAC nonetheless experience graft rejection
13^{5,6}, suggesting that whole blood trough concentration may not be the most appropriate
14 surrogate marker of pharmacodynamics (PD) in these patients. Different approaches such as
15 evaluation of TAC intracellular concentration in peripheral blood mononuclear cells (PBMC)⁷
16 or calcineurin activity (CNA) in PBMC⁶⁻⁹ could be helpful to overcome this issue in those
17 patients. However, they are not currently used for the clinical management of liver
18 transplant recipients in daily clinical practice.

19 Liver transplant recipients are usually treated with twice-daily immediate-release tacrolimus
20 (IRT) (Prograf®). Non-adherence to treatment has been found to be a significant factor
21 associated with graft rejection and graft loss¹⁰. A once-daily prolonged-release tacrolimus
22 (PRT) (Advagraf®) has been developed to improve treatment adherence. The phase III trial
23 conducted in *de novo* liver transplant recipients showed that both efficacy and safety
24 profiles were similar between twice-daily IRT and once-daily PRT¹¹. The twice-daily dosage
25 of IRT usually shifts to once-daily PRT based on a one-to-one conversion (i.e. same daily dose
26 for IRT and PRT). The narrow therapeutic range and the significant BSV in the
27 pharmacokinetics (PK) of TAC, could result in significant variations in PD in some patients,
28 possibly leading to acute graft rejection within the early post-transplantation period. In this
29 context, exploring both PK and PD of once-daily PRT at the time of conversion becomes
30 mandatory. However, the PK data of once-daily PRT in liver transplant recipients are very
31 sparse. A single population PK study was conducted to investigate once-daily PRT PK in

1 stable liver transplant recipients ¹², while another study using a standard non-
2 compartmental approach characterized its PK during the early post-transplantation period
3 ¹³. In this context, a population PK study including data from the early and late post-
4 transplantation period could be interesting to better characterize the PK/PD relationship of
5 once-daily PRT in liver transplant recipients. Finally, as far as we know, the profile of CNA has
6 not been investigated in PBMC from liver transplant recipients treated with once-daily PRT.
7 The aim of this study was to describe the PK of once-daily PRT using a population approach
8 and to characterize the CNA profile in PBMC in liver transplant recipients treated with once-
9 daily PRT and included in the CONVERSION[®] trial.

1 **2. Patients and Methods**

2 **Study population and treatment**

3 The CONVERSION[®] trial (ClinicalTrials.gov Registration identification NCT02105155) is a
4 prospective, randomized, multicenter trial aiming to prove the non-inferiority of the early
5 conversion from IRT to PRT versus the conversion at three months after liver
6 transplantation. Eligible patients (>18 years) underwent liver transplantation at day 1 (D1)
7 and started treatment with IRT (Prograf[®]). A conversion from IRT to PRT (Advagraf[®]) was
8 performed at D7 and D90 after transplantation in groups A and B, respectively (Figure 1).
9 The dosage of twice-daily IRT shifted to once-daily PRT based on a one-to-one conversion (i.e.
10 same daily dose for IRT and PRT). After conversion, daily dosing was adjusted according to
11 TAC whole blood trough concentration with a therapeutic range of 6 – 10 ng/mL ¹. All
12 patients provided written informed consent. The protocol was approved by the Committee
13 for the Protection of Persons and the French National Agency for Medicines and Health
14 Products Safety.

15 Two hundred and fifty liver transplant recipients were supposed to be included in the
16 CONVERSION[®] trial, and 40 of them in the PK/PD study ($n = 20$ in each group). However, only
17 90 patients were included in the CONVERSION[®] trial because of numerous simultaneous
18 clinical trials. Furthermore, many patients refused to participate in the PK/PD study because
19 of the lack of personal gain. In this context, PK and PD data come from 24 patients included
20 in the CONVERSION[®] trial.

21 **PK data collection**

22 Extensive PK sampling was performed at D14 post-transplantation (i.e. at D7 post-
23 conversion) in group A and at D104 post-transplantation (i.e. at D14 post-conversion) in
24 group B (Figure 1). Blood samples (7 mL) were drawn before next administration (at trough),
25 0.33, 0.66, 1, 2, 3, 4, 6, 8 and 24 hours after drug intake. Blood samples were also collected
26 right before next drug intake (trough concentration) at D5, D7, D14, D30, D90 and D180
27 post-transplantation in group A and at D90, D104 and D180 post-transplantation in group B
28 (Figure 1). Whole blood TAC concentrations were assayed using an ECLIA method ¹⁴ on
29 Cobas 8000 (Roche Diagnostics, Meylan, France). The calibration range of the ECLIA method
30 was 1 – 40 ng/mL with a limit of detection of 0.5 ng/mL. The intermediate precision and

1 accuracy of the ECLIA method were below 8.1% and 5.1%, respectively, at three levels of
2 concentrations (2.5, 10.4 and 19.8 ng/mL) ¹⁴. The accuracy of our method was ensured by
3 our participation in the TAC Proficiency Testing Scheme provided by the Cardiac and
4 Vascular Sciences Analytic Unit of St. George's Hospital Medical School (D. Holt, London,
5 United Kingdom).

6 At each follow-up visit, body composition and biological parameters were collected: body
7 weight (BW), lean body mass (LBM), hematocrit (HT), glomerular filtration rate (GFR)
8 estimated by Cockcroft-Gault formula, alanine aminotransferase (ALT), aspartate
9 aminotransferase (AST), albumin (ALB), bilirubin (BIL). LBM was estimated according to the
10 McLeay *et al.* formula ¹⁵.

11

12 **Calcineurin activity in PBMC**

13 Trough CNA in PBMC (just before drug intake) was assayed immediately before the switch to
14 PRT (i.e. at D7 and D90 post-transplant for groups A and B, respectively, Figure 1).
15 Furthermore, CNA was assayed on the blood samples from extensive PK sampling (D14 for
16 group A and D104 for group B) before next administration (at trough), 0.33, 0.66, 1, 2, 3, 4,
17 6, 8 and 24 hours after drug intake. For each blood sample, PBMC isolation was performed
18 within 24 hours after blood collection ¹⁶. First, granulocyte depletion was performed to
19 prevent the influence of granulocytes on CNA ¹⁷. For this purpose, the RosettSep[®] kit was
20 used according to the manufacturer's instructions (StemCell Technologies, Grenoble,
21 France). Second, PBMC were isolated by Ficoll density-gradient centrifugation (Unisep Ficoll-
22 tubes, Abcys, Jerusalem, Israel), then washed and counted with Xn-9000 (Sysmex, Villepinte,
23 France). Each sample including 10⁶ PBMC was dried and frozen at -80°C up to analysis. CNA
24 assay was run in duplicate as previously described ¹⁶. Briefly, PBMC lysates were incubated
25 for 15 minutes at 30°C in analysis buffer including 50 mM Tris-HCl, pH 7.0, 0.1 M Ethylene
26 glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA), 0.5 mM dithiothreitol, 1
27 mM MnCl₂, 0.3 mg/mL bovine serum albumin, 0.1 mM EGTA, 1 mM CaCl₂, 0.1 μM
28 calmodulin and 500 nM okadaic acid. The reaction was initiated by adding a 19 amino-acid
29 phosphopeptide (DLDVPIGRRFDRRVSVAEE, Bachem, Voisin, France). Aliquots were sampled
30 at 5 and 10 minutes. The reaction was stopped with 0.5% perchloric acid. Dephosphorylated
31 peptide concentrations were determined using high-performance liquid chromatography

1 coupled with UV detection. The chromatography system consisted of Dionex Ultimate 300
2 equipped with a gradient pump with degas option and gradient mixer, a UV-visible detector,
3 an autosampler, and a Chromeleon® chromatography workstation (Dionex Corporation,
4 Sunnyvale, CA, USA). The within-day precision of this method was 13.3% including all the
5 steps from blood collection to CNA assay ¹⁶. CNA was expressed as picomole of formed
6 dephosphorylated peptide per minute per 10⁶ PBMC (pmol/min/10⁶ cells).

7

8 **Non-compartmental Pharmacokinetic Analysis**

9 Whole blood concentrations of TAC from extensive PK sampling were used to calculate the
10 area under the TAC concentration-time curve over the dosing interval (AUC_{TAC}) using the
11 trapezoidal rule.

12 **Population Pharmacokinetic Analysis**

13 TAC concentration-time data were analyzed by nonlinear mixed effects modeling using
14 NONMEM® software (version 7.4, ICON Development Solutions, Ellicott City, MD, USA) with
15 Piraña® (version 2.9.7) and PsN toolkit (version 4.7.0). Analyses were carried out with first
16 order conditional estimation method with interaction (FOCE-I). Data processing and plots
17 were performed in R (version 3.4.2). Several structural models were used to fit the
18 concentration-time data. First, one and two compartment models with first order absorption
19 and elimination were tested. Since TAC was administered as a prolonged-release
20 formulation, a first order process with either a lag time or transit compartments with an
21 identical transfer rate constant (k_{tr}) were tested to account for the delay in the absorption
22 phase. The inclusion of BSV and between-occasion variability (BOV) defined as $OCC_1 \leq D28$
23 and $OCC_2 > D28$ for group A and $OCC_1 \leq D105$ and $OCC_2 > D105$ for group B was tested on all
24 PK parameters according to an exponential model:

$$25 \quad \theta_i = \theta_\mu \cdot \exp(\eta_i + \eta_{1i}OCC_1 + \eta_{2i}OCC_2)$$

26 where θ_i is the estimate of the parameter for the i th subject, θ_μ is the population mean
27 estimate of the PK parameter, η_i is the deviation from the mean for the i th subject with zero
28 mean and variance ω^2 , η_{1i} and η_{2i} is the deviation from the mean for the first (OCC_1) and
29 second (OCC_2) occasion for the i th subject, respectively. Correlation between η of PK
30 parameters was tested using a ω block structure. The residual unexplained variability was
31 described using a proportional error model. Model selection was based on the objective

1 function value (OFV = $-2\log\text{likelihood}$), using the likelihood ratio test to test for significant
2 differences in goodness-of-fit (GOF) between nested models. A drop of at least 3.84 (χ^2 test,
3 $\alpha = 5\%$, degree of freedom = 1) between hierarchical models was considered statistically
4 significant. Additionally, the plausibility of parameter estimates with their precision
5 (expressed by relative standard error, %RSE), η -shrinkage value and model stability were
6 considered.

7 *Covariate analysis*

8 The individual parameter estimates of the base model were used to investigate the
9 correlations with biological and demographic variables. The following covariates were tested
10 for their influence on clearance (CL): age, sex (0 for male and 1 for female), BW, LBM, HT,
11 GFR, AST, ALT, ALB, BIL and study group (GRP). As PK data come from a large time period,
12 different values of a covariate for the same patient were included in the data. Continuous
13 covariates were tested according to the linear function:

$$14 \quad CL = \theta_{CL} \times (1 + \theta_{cov} \times (COV - COV_{mean}))$$

15 where θ_{CL} is the typical value of CL in the population, cov is the individual covariate value,
16 COV_{mean} is the mean value of a covariate in the studied population, θ_{cov} is the fractional
17 change in CL from the mean value of the covariate. Categorical covariates (sex, study group)
18 were tested according to the following equation:

$$19 \quad CL = \theta_{CL} \times \theta_{cov}^{COV}$$

20 where θ_{cov} is the estimated influential factor for a covariate and cov is 1 or 0. In the forward
21 procedure, covariates were tested one by one and a covariate was considered significantly
22 associated with a PK parameter if its inclusion resulted in a drop in OFV of at least 3.84
23 points (χ^2 test, $\alpha = 5\%$, $df = 1$). In the backward procedure, a full covariate model including
24 the covariates significant in the forward procedure was built. A covariate remained in the
25 final model if its removal resulted in an increase of at least 6.63 points (χ^2 test, $\alpha = 1\%$, $df =$
26 1) compared to the full covariate model.

27 *Model evaluation*

28 Diagnostic plots including population predictions (PRED) versus observed concentrations
29 (DV), individual predictions (IPRED) versus DV, conditional weighted residuals (CWRES)

1 versus PRED and time after dose were generated. Since patients were treated with different
2 doses of TAC, the model validation was performed with a prediction-corrected visual
3 predictive check (pcVPC) based on 1000 replicates of the original data set and presented as
4 concentrations versus time after dose and stratified on study group to facilitate
5 interpretation. Finally, 500 bootstrap analyses with resampling using the final model were
6 performed.

7 *Analysis of the individual PK parameters*

8 The individual CL values obtained in NONMEM were used to calculate AUC_{TAC} according to
9 the following formula in the model input file:

$$10 \quad AUC_{ij} = DOSE_i \times F/CL_{ij}$$

11 where AUC_{ij} is the area under the concentration-time curve over the dosing interval for the
12 i th subject and j th occasion, $DOSE_i$ is the administered dose for the i th subject, CL_{ij} is the
13 individual clearance value for the i th subject and j th occasion and F is the oral bioavailability
14 of TAC (fixed in the model to 0.23 based on the literature)¹⁸.

15 **Statistical Analysis**

16 The demographic and biological characteristics of the study cohort are presented as median
17 [interquartile range]. PK data are expressed as geometric mean [95% confidence interval,
18 CI95%] and PD data are expressed as mean \pm SD. The individual AUC_{TAC} values obtained by
19 non-compartmental analysis and population approach were normalized by the median daily
20 dose which was administered prior to extensive PK sampling. Individual AUC_{TAC} obtained in
21 the non-compartmental analysis were compared with model-predicted AUC_{TAC} for group A
22 and B using non-parametric Wilcoxon paired sample test. AUC_{TAC} obtained by both non-
23 compartmental analysis and population approach were compared between group A and B
24 using a Wilcoxon unpaired samples test. Since the number of patients per each study group
25 is low, the ratio of the geometric means of AUC_{TAC} group A over AUC_{TAC} group B as well as its
26 CI90% was calculated in addition to the non-parametric statistical tests to compare AUC_{TAC}
27 between groups A and B.

28 From data of extensive PK sampling, individual 24-hour area under the calcineurin activity
29 versus time curve (AUC_{CNA}) was calculated using the trapezoidal rule. Only PD data from

1 extensive PK sampling were used to investigate the PK/PD relationship. The AUC_{CNA} were
2 compared between groups A and B using a Wilcoxon unpaired samples test. The relationship
3 between AUC_{TAC} and AUC_{CNA} was tested using Spearman's correlation test. All tests were
4 two-sided, and they were considered significant when p-values were <0.05 . Computations
5 were performed using R software and SAS V9 statistical package (SAS institute, Cary, NC,
6 USA).

1 3. Results

2 Patients and TAC concentrations

3 The baseline demographic and biological characteristics of 24 patients ($n = 12$ patients in
4 each group) included in the study are summarized in Table 1. Overall, 221 blood samples
5 including those from therapeutic drug monitoring were available for the PK analysis. The
6 median number of measurements per individual was 11 (range 1 – 13). The sampling time
7 was in the range 0.1 – 27 h after drug intake. Four patients who did not have extensive PK
8 sampling ($n = 1$ in group A and $n = 3$ in group B) withdrew their informed consent on the day
9 of the analysis as they did not understand that a part of the study included several blood
10 samples drawn throughout the day and required them to stay for a longer time in the
11 medical department. For the remaining patients ($n = 20$), extensive PK sampling was
12 performed at median 14 days (range 13 – 21) and 104 days (95 – 109) after transplantation
13 in groups A and B, respectively. Figure 2 presents TAC concentrations versus time after dose
14 at D14 ($n = 11$) and D104 ($n = 9$) for groups A and B, respectively (data from extensive PK
15 sampling only).

16

17 Non-compartmental Pharmacokinetic Analysis

18 AUC_{TAC} values were calculated using trapezoidal rule for the 20 patients ($n = 11$ and $n = 9$ for
19 groups A and B, respectively) for which extensive PK data were available. The absolute
20 AUC_{TAC} means obtained by the non-compartmental analysis were similar between groups A
21 and B (251.3 ng/mL.h [CI95% = 108.5 – 460.7] and 200.7 ng/mL.h [CI95% = 126.0 – 302.2],
22 respectively, $p = 0.17$).

23 At the time of extensive PK sampling, the median dose of PRT was 7.0 mg/day and 5.0
24 mg/day in groups A and B, respectively, whereas median dose was 6.0 mg/day regardless of
25 study group. The geometric means of dose-normalized AUC_{TAC} (6.0 mg/day) were similar in
26 groups A and B (234.5 ng/ml.h [CI95% = 130.3 – 670.6] and 231.0 ng/ml.h [CI95% = 120.2 –
27 433.4], respectively). The ratio of the geometric means of $AUC_{TAC\ group\ A}$ over $AUC_{TAC\ group\ B}$ was
28 1.01 [CI90% = 0.66 – 1.56] (Table 2). The dose-normalized AUC_{TAC} obtained by non-
29 compartmental analysis were not statistically different between groups A and B ($p = 0.77$).

1 **Population Pharmacokinetic Analysis**

2 TAC concentration-time data were described by a two-compartment model with linear
3 elimination and a delayed first order absorption characterized by two transit compartments
4 with an identical k_{tr} . Addition of transit compartments to describe the absorption phase
5 resulted in a significant improvement of the model fit: one transit compartment dropped
6 OFV by 14 points and two transit compartments by 25 points compared to the model
7 without delayed absorption. Further addition of a third transit compartment did not improve
8 the model fit. The PK parameters of the final model were: k_{tr} , clearance (CL), volume of
9 distribution of the central compartment (V_c), inter-compartmental clearance (Q), volume of
10 distribution of the peripheral compartment (V_p). The bioavailability (F) of TAC was fixed to
11 the value previously reported in the literature ($F = 0.23$)¹⁸. Therefore, the PK parameters
12 (CL, V_c , Q, V_p) were reported as absolute values. BSV was included on k_{tr} , CL and Q. BSV could
13 not be reliably estimated on V_c and V_p and inclusion of BSV on F did not improve the model
14 fit, thus BSV was fixed to zero for these three parameters. The addition of covariance
15 between η of the PK parameters did not improve the model fit. Finally, inclusion of BOV on
16 CL resulted in a drop of 86 points in OFV and decreased the residual variability from 27.8% to
17 19.8%.

18 *Covariate analysis*

19 The covariate analysis was performed on CL only as $\eta_{k_{tr}}$ showed significant deviation from a
20 normal distribution (Shapiro-Wilk test, $p = 0.02$) and η_Q was associated with shrinkage of
21 35%. The correlation plots between individual CL of OCC₁ and OCC₂ and continuous
22 covariates are presented in Supplementary Figure 1. The lack of influence of sex and GRP on
23 CL is presented in Supplementary Figure 2. In the forward analysis, none of the tested
24 covariates was significantly associated with CL (Supplementary Table 1) thus the final model
25 did not include covariates. The estimates of the final model with corresponding %RSE are
26 presented in Table 3.

27 *Evaluation of the final model*

28 GOF plots depicted in Figure 3 show no major bias of the model based on IPRED vs DV plot
29 whereas CWRES vs PRED and time after dose were homogeneously distributed around the
30 zero line although a slight bias at higher PRED values was observed. The pcVPC showed that

1 the 5th, 95th percentiles and the median of the simulated data are in good agreement with
2 the 5th and 95th percentiles and the median of the observed concentrations for both groups
3 A and B (Figure 4). Finally, the mean estimates of the PK parameters from 500 bootstrap
4 analyses are in accordance with those estimated using the original data set (Table 3).

5 *Analysis of individual PK parameters*

6 Model-predicted absolute AUC_{TAC} at extensive PK sampling (corresponding to OCC₁ for both
7 group A and B) was not statistically different between group A and B (252.4 ng/mL.h [CI95% =
8 111.3 – 510.5] vs 195.2 ng/mL.h [CI95% = 124.9 – 302.1], respectively, $p = 0.17$, $n = 20$).
9 Table 2 presents model-predicted geometric means of AUC_{TAC} normalized for a median dose
10 of 6.0 mg/day. Dose-normalized AUC_{TAC} were not statistically different between groups A
11 and B (235.6 ng/mL.h [CI95% = 139.6 – 598.7] and 224.6 ng/mL.h [CI95% = 117.6 – 421.5]
12 ng/mL.h, respectively, $p = 0.94$) and the ratio of the geometric means of AUC_{TAC group A} over
13 AUC_{TAC group B} was 1.05 [CI90% = 0.70 – 1.57] (Table 2). Finally, the comparison of AUC_{TAC}
14 obtained either by non-compartmental analysis or by population approach showed that
15 both values were similar ($p = 0.90$ and $p = 0.25$ for groups A and B, respectively) further
16 validating our PK model.

17

18 **PRT pharmacodynamics**

19 Figure 5 presents individual CNA profile (log scale) over the dosing interval at D14 for group
20 A ($n = 11$) and D104 for group B ($n = 9$). The AUC_{CNA} means were not statistically different
21 between groups A and B (4897 ± 3437 and 4079 ± 1008 pmol/min/10⁶ cells, Wilcoxon
22 unpaired t-test $p = 0.50$). However, a larger BSV in AUC_{CNA} was observed in group A (70.2 vs
23 24.7% for groups A and B, respectively). No relationship was found between AUC_{CNA} and
24 either model-predicted absolute AUC_{TAC} (rho coefficient, $\rho = 0.26$, [CI95% = -0.20; 0.63]; $p =$
25 0.25 ; Figure 6) or TAC whole blood trough concentration (rho coefficient, $\rho = 0.20$, [CI95% = -
26 0.27 ; 0.59], $p = 0.39$). The mean trough CNA activity (just prior TAC intake) at D14 post-
27 transplantation in group A was statistically higher than that measured just before the switch
28 to PRT (i.e. D7 post-transplantation) (198 ± 92 vs 124 ± 72 pmol/min/10⁶cells, $n = 8$,
29 respectively; paired t-test, $p = 0.048$), while no statistical difference was observed for TAC
30 whole blood trough concentration (6.9 ± 2.3 vs 10.1 ± 5.4 ng/mL, respectively; paired t-test,
31 $p = 0.11$). Finally, no statistical difference between D90 and D104 was observed for either

- 1 trough CNA (149 ± 78 vs 172 ± 82 pmol/min/ 10^6 cells, $n = 6$; paired t-test, $p = 0.18$) or TAC
- 2 whole blood trough concentration (8.8 ± 4.6 vs 5.9 ± 2.1 ng/mL, respectively; paired t-test, p
- 3 = 0.17). Finally, no graft rejection was observed in either group.

1 4. Discussion

2 PRT (Advagraf®) is EMA-approved for use in the context of liver transplants. However, there
3 is limited published data regarding the PK and PD of PRT in this indication. As far as we
4 know, the present study is the first to assess the PK of PRT within the early and late post-
5 transplantation periods using a population approach. Furthermore, it provides new insights
6 about the profile of CNA in PBMC from liver transplant recipients treated with PRT.

7 In the population PK analysis, blood concentration-time data of once-daily PRT were
8 described by a two-compartment model with delayed absorption characterized by two
9 transit compartments. This is consistent with a previous population PK study reported by
10 Moes *et al.* in which a two-compartment model with three transit compartments was used
11 to characterize the PK of once-daily PRT in 66 stable liver transplant recipients¹². The mean
12 estimate of CL in our analysis was 5.1 L/h (BSV = 34.7%) which is close to the value reported
13 by Moes *et al.* (4.77 L/h, BSV = 45.4%). The analysis of the demographic and biological
14 covariates on CL did not allow us to identify any significant correlations. This may be due to
15 small sample size and the small dispersion of the covariates in our study. Nevertheless, in
16 stable liver transplant recipients treated with PRT, Moes *et al.* did not report any significant
17 influence of the covariates which we tested on total CL¹².

18 It has been reported that the expression of *CYP3A5*1*, both in donor and receiver of a liver
19 transplant, significantly increases CL of TAC in patients treated with PRT¹². Other studies
20 conducted in kidney transplant recipients treated with PRT also reported the influence of
21 *CYP3A5*1* on CL^{19,20}. We could not confirm or contradict these results because
22 pharmacogenetic data were not available in our study. It was decided not to conduct an
23 analysis of *CYP3A5*1* genotype in the CONVERSION study because the frequency of
24 *CYP3A5*1* genotype in the French population is low (13%)²¹ and as the study included a
25 small number of patients, the statistical power would not have been sufficient to draw any
26 firm conclusion. Similarly, using a mixture model in the PK population analysis to identify the
27 subpopulation carrying the *CYP3A5*1* allele would not have been possible. Regarding the
28 genetic polymorphisms of drug transporters such as MDR1, although its influence on TAC PK
29 has been reported, the results still remain controversial²². In the same way as for the
30 *CYP3A5*1* genotype, our study could not contribute any results regarding the impact of

1 genetic polymorphisms of drug transporters on TAC PK because of the lack of statistical
2 power.

3 To further evaluate the validity of our model, the individual AUC_{TAC} obtained using a
4 population approach were compared with those obtained using a non-compartmental
5 analysis with data from extensive PK sampling. AUC_{TAC} means of groups A and B obtained
6 using either a non-compartmental or a population approach were not statistically different
7 ($p = 0.90$ and $p = 0.25$ for groups A and B, respectively). Furthermore, comparison of AUC_{TAC}
8 values obtained by both approaches showed no statistical differences between groups A and
9 B ($p = 0.77$ and $p = 0.94$, respectively). Finally, the geometric means of model-predicted
10 dose-normalized AUC_{TAC} in our study (235.6 ng/mL.h [CI95% = 139.6 – 598.7] and 224.6
11 ng/mL.h [CI95% = 117.6 – 421.5] for groups A and B, respectively, normalized to median
12 dose of 6.0 mg/day) are close to those previously reported in liver transplant recipients
13 obtained using a non-compartmental approach²³. Indeed, Florman *et al.* reported a mean
14 AUC_{TAC} of 184 ± 63 ng.h/mL at day 28 post-transplantation in liver transplant recipients
15 treated with PRT (mean dose of 5.2 mg/day). Taken together, these results suggest that the
16 developed model satisfyingly describes the TAC concentration-time data. However, the
17 limitation of our PK analysis is the small number of patients. Therefore, our results are not
18 conclusive and need to be confirmed in larger cohorts. Moreover, some individual PK
19 profiles in our study show a second peak of absorption. This was previously observed in liver
20 and kidney transplant recipients treated with a different PRT formulation (Envarsus®)^{24,25}
21 and was described by a double-gamma absorption model. In our analysis, the attempts to
22 describe the second absorption peak did not give a reliable estimation of the PK parameters
23 probably due to an insufficient number of samples in the absorption phase or the fact that it
24 was only observed in some patients. In addition, the low number of PK samples in the
25 absorption and distribution phases might be the reason for high BSV on k_{tr} and Q. Although
26 we analyzed the PK data with a model which did not account for the second peak of
27 absorption, the comparison of AUC_{TAC} values obtained with the non-compartmental
28 approach and predicted by the PK model were in good agreement for both study groups
29 which shows that our model accurately described the data.

30 CNA is a surrogate marker of TAC PD. Different PK/PD studies conducted in liver transplant
31 recipients have suggested that assessment of CNA within the early post-transplantation
32 period could be helpful to predict acute graft rejection in patients well exposed to TAC^{6,7}. In

1 the present study, no relationship was found between AUC_{TAC} and AUC_{CNA} values regardless
2 of the moment of conversion from IRT to PRT, as previously reported⁶⁻⁸. Different factors
3 such as the amount of cytosolic FKBP12²⁶ and FKBP13, FKBP51 acting as a reservoir², the
4 genetic polymorphism of the calcineurin catalytic subunit α ^{27,28} and the etiology of liver
5 disease before transplant²⁹ might significantly influence the CNA in PBMC regardless of the
6 whole blood TAC concentration. Besides, Lemaitre *et al.* showed that CNA in PBMC was not
7 further associated to intracellular concentration of TAC in liver transplant recipients⁷, which
8 supports our result. The BSV in AUC_{CNA} for group A is in accordance with that reported at D7
9 and D14 post-transplantation in liver transplant recipients treated with twice-daily IRT⁶⁻⁸.
10 However, it was 3-fold higher compared to the BSV in AUC_{CNA} for group B (70.2 vs 24.7%,
11 respectively) while AUC_{CNA} means were not statistically different in both groups. In addition,
12 absolute AUC_{TAC} values were similar between groups A and B ($p = 0.17$) which altogether
13 suggests that factors other than drug exposure contribute to this variability. Although
14 patients' characteristics regarding immunophilins (FKBP12, 13 and 51) were probably
15 different between both groups, the magnitude of immune response during the early post-
16 transplantation period might also contribute to the large BSV in AUC_{CNA} . Besides, our study
17 shows that the conversion from IRT to PRT in a 1:1 ratio based on total mg/day dose could
18 also contribute to this variability. Interestingly, trough CNA at D14 in group A was
19 statistically higher than that measured just before the switch to PRT ($p = 0.048$), while no
20 difference in TAC whole blood trough concentration was observed. Furthermore, neither
21 trough CNA nor TAC whole blood trough concentration at D90 and D104 was different in
22 group B. Finally, no graft rejection was observed in our PK/PD study regardless of study
23 group. Although the number of patients was limited, these results suggest that the
24 conversion from IRT to PRT during the early post-transplantation period could modify PD
25 profile of calcineurin without causing graft rejection. Further investigations with a larger
26 cohort of patients should be conducted to confirm this result.

27 In conclusion, we have developed a population PK model for PRT in order to evaluate the
28 PK/PD relationship for TAC in early and stable liver transplant recipients. The results suggest
29 that one-to-one dosage conversion from twice-daily IRT to once-daily PRT during the early
30 post-transplantation period could modify CNA in PBMC which might not be related to TAC
31 PK. The advantage of our study is the PK and PD comparison between early and stable
32 transplant recipients. Using both a non-compartmental analysis and a population approach,

1 we showed that the mean AUC_{TAC} values between group A and B were not statistically
2 significantly different. Therefore, the model we have developed can be used to predict TAC
3 whole blood concentrations in liver transplant recipients under the same conditions and
4 dosing regimen as specified in our study. However, as the sample size in our study is low, our
5 results should first be confirmed in larger cohorts.

6

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10 Research Unit of East of Paris (URC-Est), Saint Antoine University Hospital (AP-HP) for study
11 coordination and logistics.

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- 28
- 29

1 **Figure Legends**

2

3 **Figure 1.** Design of the pharmacokinetic/pharmacodynamic study in the CONVERSION® trial.

4

5 **Figure 2** Individual pharmacokinetic profiles of once-daily prolonged-release tacrolimus from
6 extensive sampling day, corresponding to day 14 for group A ($n = 11$) and day 104 for group
7 B ($n = 9$).

8

9 **Figure 3.** Goodness-of-fit plots of the final model. (PRED, population predictions, IPRED,
10 individual predictions, DV, observed concentrations, CWRES, conditional weighted
11 residuals).

12

13 **Figure 4.** Prediction-corrected visual predictive check stratified on study group based on
14 1000 replicates of the original data set using the final model. *Blue lines* represent the 5th
15 and 95th percentiles of the observed concentrations, *red line* represents the median of the
16 observed concentrations, *blue areas* represent 95% confidence intervals around 5th and
17 95th percentiles of the simulated concentrations, *red area* represents 95% confidence
18 interval around the median of the simulated concentrations and *black points* represent
19 observed concentrations.

20

21 **Figure 5.** Individual CNA profile over the dosing interval at day 14 for group A ($n = 11$) and
22 day 104 for group B ($n = 9$).

23

24 **Figure 6.** Relationship between area under the tacrolimus concentration-time curve over the
25 dosing interval (AUC_{TAC}) and 24-hour area under the calcineurin activity curve (AUC_{CNA}) in
26 liver transplant recipients treated with once-daily prolonged-release tacrolimus. Calcineurin
27 activity (CNA) is expressed for 10^6 cells.

28

1 **Supplementary Material**

2

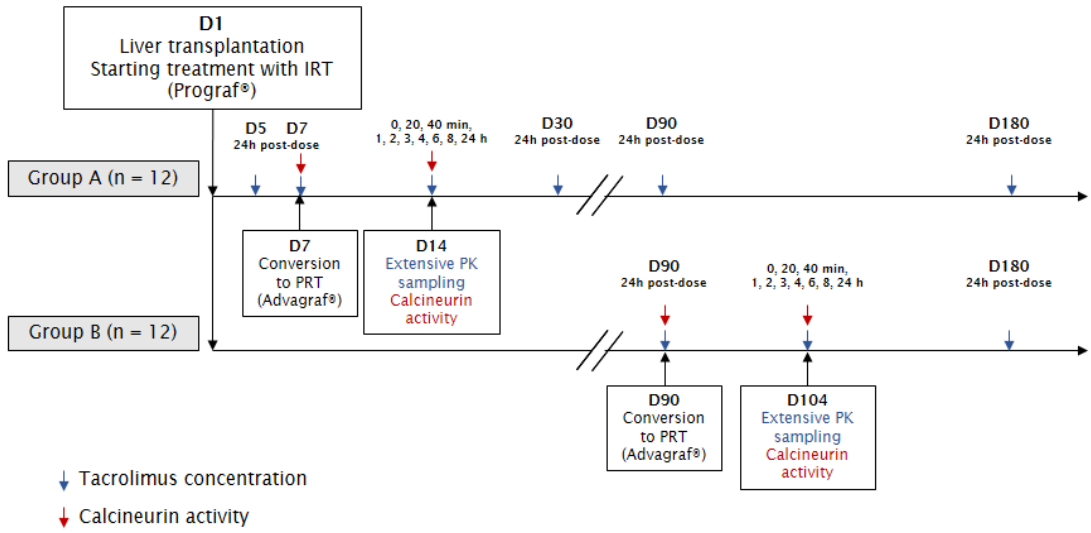
3 **Supplementary Table 1** Results of the covariate analysis using the base model (forward
4 step).

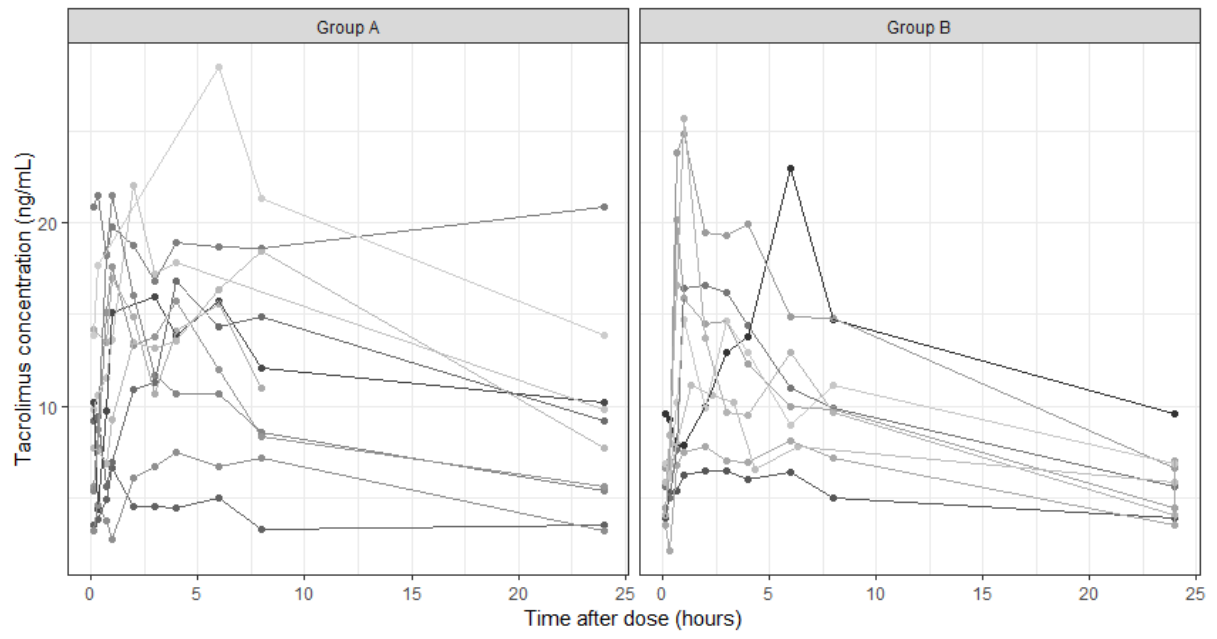
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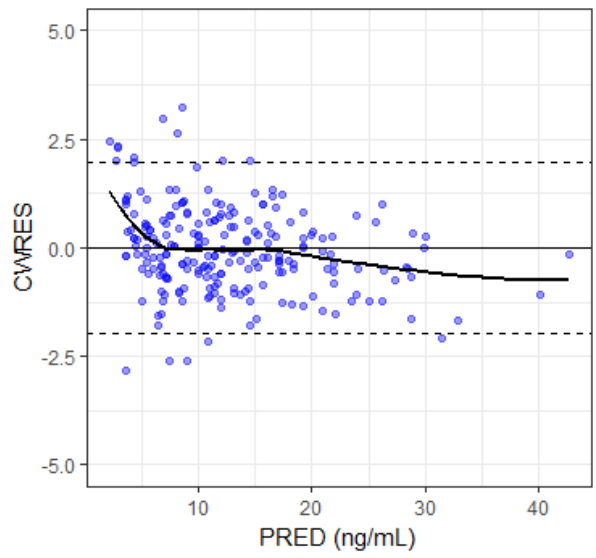
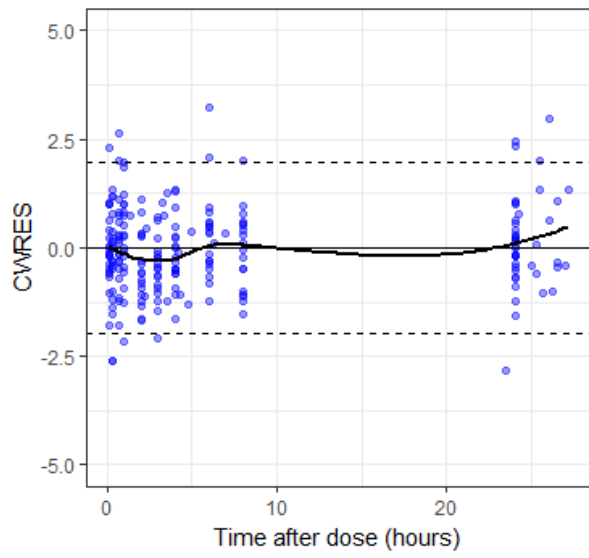
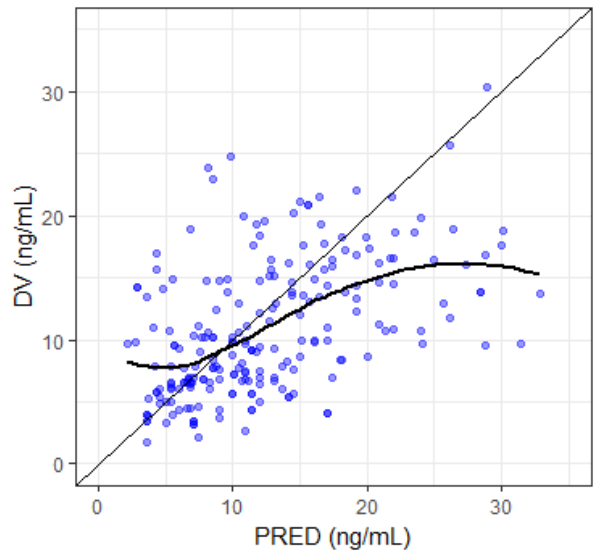
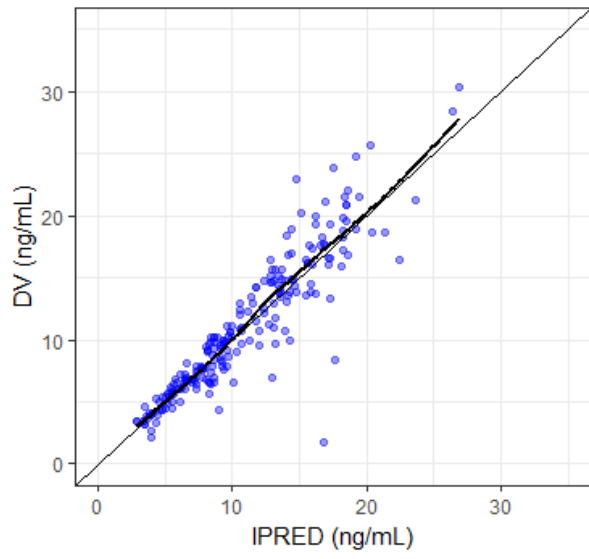
6 **Supplementary Figure 1** (a) Correlation plots between individual absolute clearance (CL)
7 obtained from the population approach and continuous covariates at the first
8 pharmacokinetic occasion ($OCC_1 \leq 28$ days for group A and $OCC_1 \leq 105$ days for group B); (b)
9 Correlation plots between individual absolute clearance (CL) obtained from the population
10 approach and continuous covariates at the second pharmacokinetic occasion ($OCC_2 > 28$
11 days for group A and $OCC_2 > 105$ days for group B).

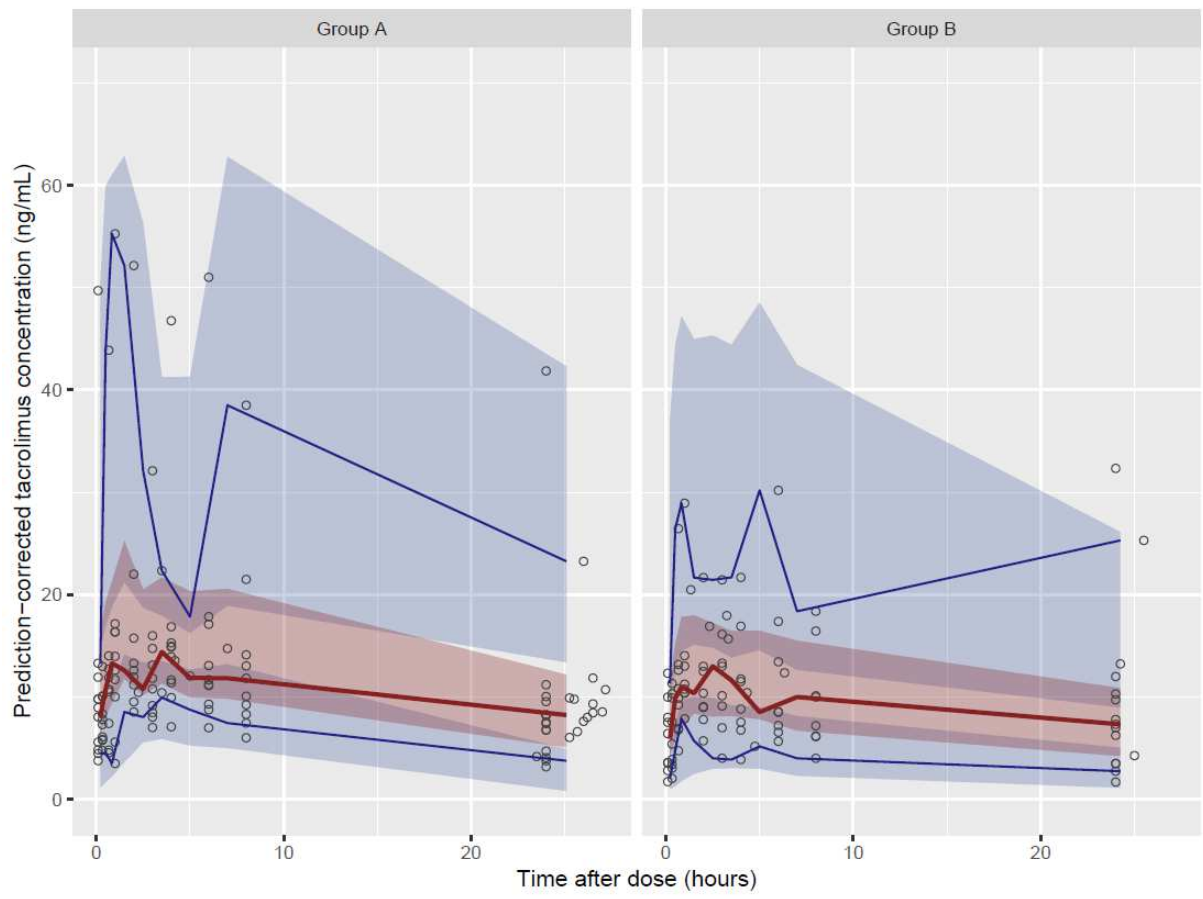
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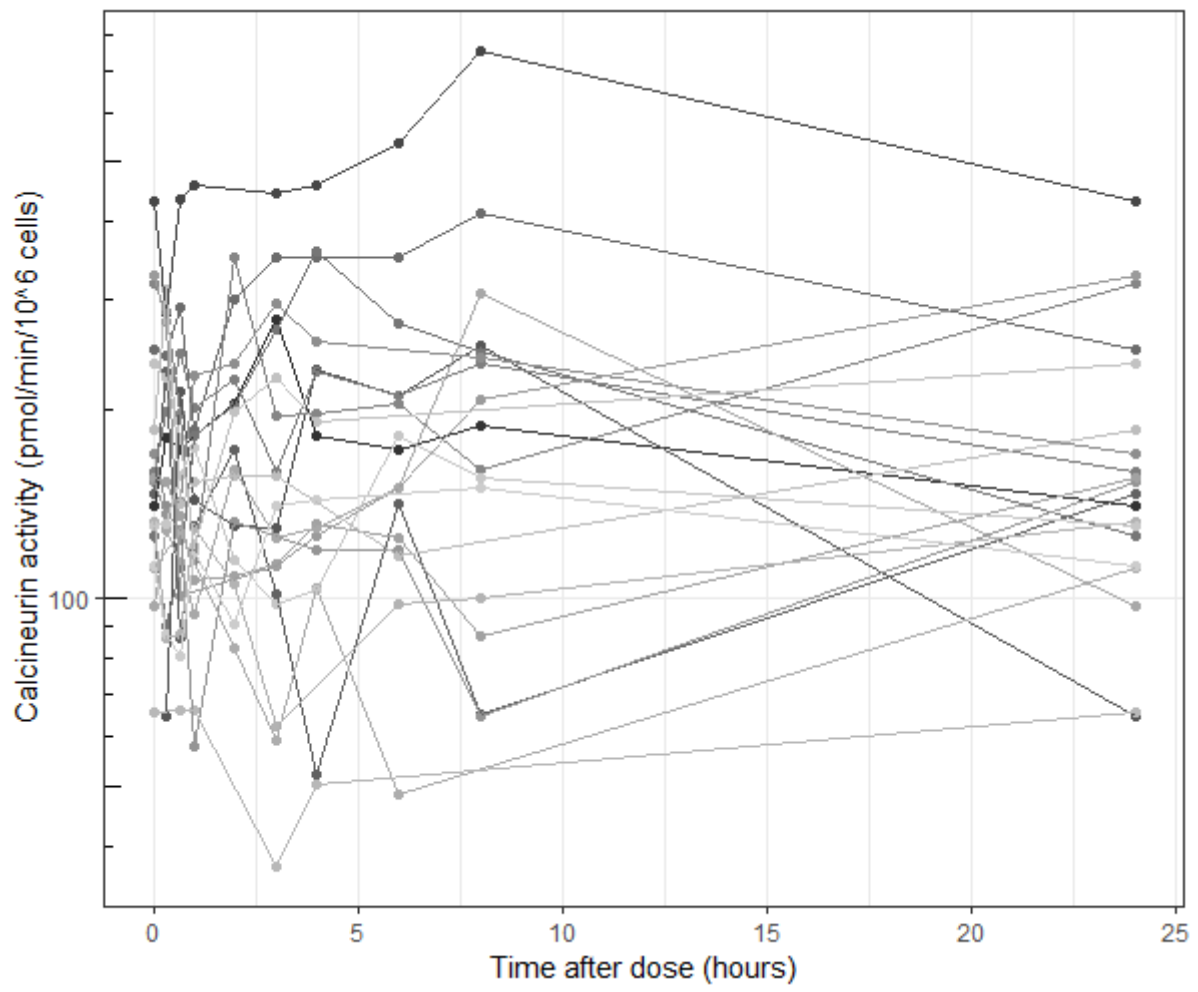
13 **Supplementary Figure 2** Box-plots for individual absolute clearance (CL) obtained from the
14 population approach and categorical covariates.











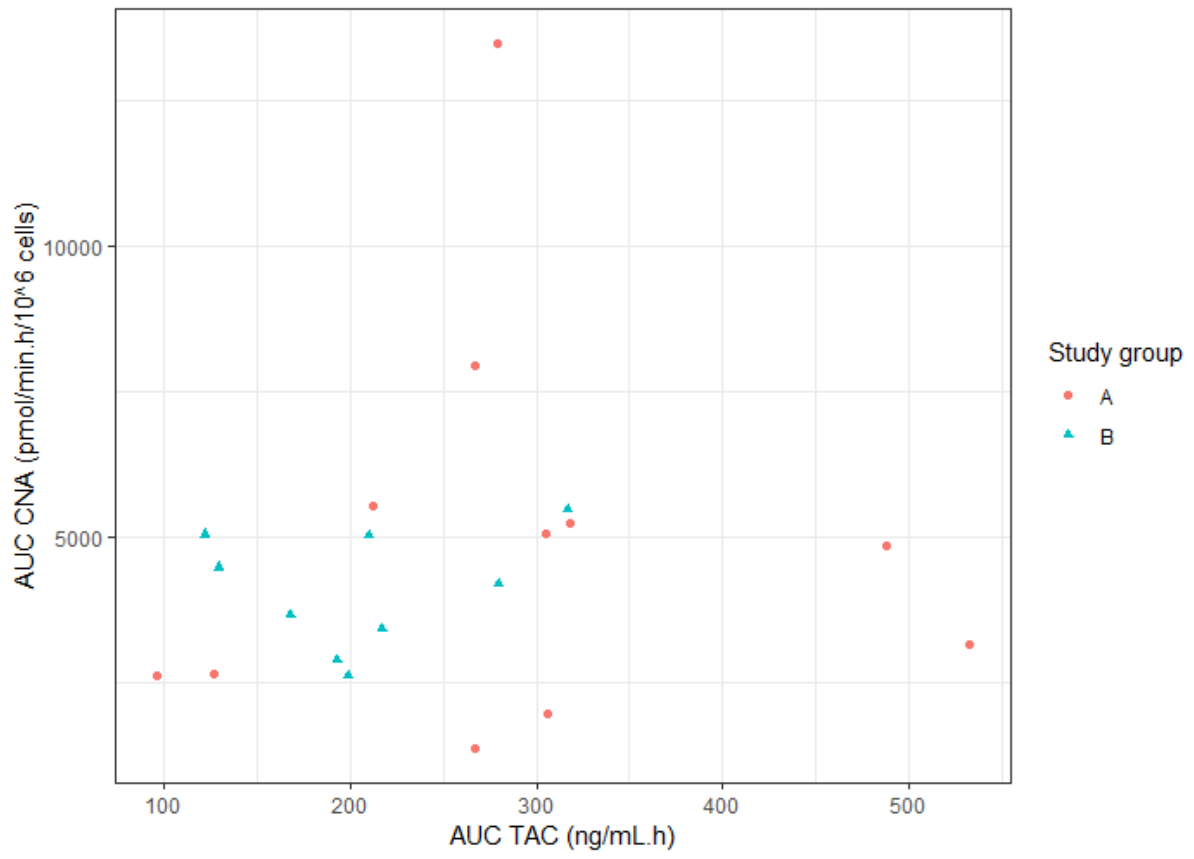


Table 1 Baseline demographic and biological characteristics of group A and B. Results are presented as median [interquartile] or median (range).

	Group A (n = 12)	Group B (n = 12)
Sex (female/male)	3/9	1/11
Age (years)	57 [53-60]	59 [54-62]
Body weight (kg)	81 [69-86]	74.0 [67-81]
Lean body mass (kg)	15 [13-16]	15 [13-15]
Biological data		
Hematocrit (%)	31 [29-33]	38 [34-39]
GFR (mL/min)	105 [70-117]	82 [54-91]
AST (UI/L)	43 [16-69]	21 [17-25]
ALT (UI/L)	85 [26-125]	11 [9-26]
Albumin (g/L)	30 [28-34]	40 [36-47]
Bilirubin (μmol/L)	24 [17-54]	8.0 [7 – 11]
Tacrolimus therapy		
Median dose ^a (mg/day)	7.0 (2.0-20.0)	5.0 (2.5-12.0)
Trough concentration (ng/mL) ^a	8.5. [5.4-10.2]	5.6. [4.1-6.6]

AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; GFR, Glomerular filtration rate

^a Median value at the time of extensive pharmacokinetic sampling

Table 2 Comparison of AUC_{TAC} obtained by non-compartmental analysis and population pharmacokinetic approach.

	AUC _{TAC} (ng/mL.h) ^a Geometric mean [CI95%]		p-value ^b	AUC _{TAC} Group A /AUC _{TAC} Group B Ratio of geometric means [CI90%]
	Group A (n = 11)	Group B (n = 9)		
Non-compartmental	234.5 [130.3 – 670.6]	231.0 [120.2 – 433.4]	0.77	1.01 [0.66 – 1.56]
Model-predicted	235.6 [139.6 – 598.7]	224.6 [117.6 – 421.5]	0.94	1.05 [0.70 – 1.57]
p-value ^c	0.90	0.25	NA	NA

CI, confidence interval; AUC_{TAC}, area under the tacrolimus concentration-time curve over the dosing interval; NA, not available

^a Dose-normalized for median daily dose of 6.0 mg

^b Wilcoxon unpaired test comparing non-compartmental and model-predicted AUC between group A and B

^c Wilcoxon paired test comparing non-compartmental and model-predicted AUC for each study group

Table 3 Mean pharmacokinetic parameter estimates obtained from the final model and from 500 bootstrap runs with resampling.

Parameter	Mean estimate (%RSE) [shrinkage]	Bootstrap mean (95% CI)
k_{tr} (h^{-1})	2.19 (19.7%)	2.14 (1.37 – 2.92)
CL (L/h)	5.09 (8.2%)	5.11 (4.36 – 5.93)
V_c (L)	93.5 (41.0%)	86.9 (54.1 - 126)
Q (L/h)	42.0 (46.2%)	43.1 (20.2 - 71.9)
V_p (L)	135 (21.0%)	142 (88.4 - 196)
F (fixed)	0.23	0.23
Between-subject variability		
k_{tr} (CV%)	94.5% (22.1%) [13.3%]	80.6% (51.6 - 111)
CL (CV%)	34.7% (31.5%) [26.1%]	33.8% (13.5 – 48.4)
Q (CV%)	151% (37.6%) [34.6%]	120% (67– 170)
Between-occasion variability ^a		
CL (CV%)	39.8% (21.3%)	36.2% (22.8 – 46.6)
Proportional error (%)	19.8% (8.6%) [14.3%]	18.7% (16.0 – 21.1)

CI, confidence interval; CL, clearance; CV, coefficient of variation; F, bioavailability; k_{tr} , transfer rate constant between transit compartments; Q, inter-compartmental clearance; RSE, relative standard error; V_c , volume of distribution of the central compartment; V_p , volume of distribution of the peripheral compartment

^a occasions (OCC) defined as: OCC1 \leq 28 days and OCC2 $>$ 28 days (group A); OCC1 \leq 105 days and OCC2 $>$ 105 days (group B)